Remarks

Claims 29, 32, 35, and 39-51 were pending in the subject application. By this Amendment, claims 29 and 43 have been amended, and claims 41-42 and 48-49 have been cancelled. The undersigned avers that no new matter is introduced by this amendment. Entry and consideration of the amendments presented herein is respectfully requested. It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of the applicants' agreement with or acquiescence in the Examiner's position. Accordingly, claims 29, 32, 35, 39, 40, 43-47, 50, and 51 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

Submitted herewith is a Request for Continued Examination (RCE) under 37 C.F.R. §1.114 for the subject application.

Claim 29 has been amended to recite that the human stem cells are embryonic stem cells or hematopoietic stem cells. Claim 43 has been amended to recite that the mouse stem cells are embryonic stem cells or hematopoietic stem cells. Support for the amendments to claims 29 and 43 can be found, for example, at page 30, lines 16-18 of paragraph [0065]; page 4, paragraphs [0010] and [0011], of the subject specification, and the claims as originally filed.

The applicants gratefully acknowledge the Examiner's indication that the rejection under 35 U.S.C. §112, first paragraph, for lack of written description, has been withdrawn in view of the state of the art of RNAi at the application's filing date.

Claims 29, 32, 35, and 39-51 are rejected under 35 U.S.C. §112, first paragraph, as non-enabled. The applicants respectfully traverse and submit that the claimed invention is fully enabled by the subject specification.

As indicated above, by this Amendment, the applicants have amended claims 29 and 43 to recite that the stem cells are embryonic stem cells or hematopoietic stem cells. Claims 41 and 48 have been cancelled. The methods of the claimed invention as currently amended are reasonably enabled by the specification, as one of ordinary skill in the art would be able to make and use the invention without undue experimentation.

At page 4, the Office Action indicates that the gene knockout data in the manuscript entitled "SHIP-deficiency enhances HSC Proliferation and Survival but Comprises Repopulating Potential" (submitted as Exhibit B with the applicants' previous response dated January 9, 2006), which was obtained using a Cre-lox mutation strategy, does not support or correlate with the use of RNAi for s-SHIP/SIP-110 gene silencing, as taught in the patent application. Specifically, the Office Action raises issues pertaining to: (1) whether one skilled in the art at the time the application was filed would reasonably expect that treating hematopoietic stem cells (HSC) with shRNA targeting s-SHIP/SIP-110 mRNA would result in HSC proliferation, as taught in the subject application; and (2) the level of RNAi-mediated gene silencing that is required such that a phenotype of expansion would occur (page 4, lines 15-19). The applicants have addressed these issues in that order.

Submitted herewith for the Examiner's consideration is a Declaration by Dr. William G. Kerr under 37 C.F.R. §1.132, accompanied by several supporting documents. As explained by Dr. Kerr in his Declaration, the full-length hematopoietic isoform of the SH2-containing inositol 5'phosphatase (SHIP), as a key signaling component and regulator of cellular responses in the mature cells of several hematopoietic lineages, is relevant to the enablement of the claims. As indicated in Dr. Kerr's previous Declaration dated January 9, 2006, it has been known for 7-8 years that SHIP opposes phosphatidylinositol 3-kinase (PI3K) and thus, PI3K-effector pathways, which control cell proliferation and/or survival. Engagement of receptors on the surface of mammalian cells results in the activation of PI3K and phosphorylation of phosphatidylinositols on the cytoplasmic side of the cell membrane. The generation of phosphatidylinositol 3,4,5-triphosphate (PIP3) by PI3K contributes to the activation of signaling pathways that drive cell proliferation. SHIP can associate with various adapter proteins, scaffold proteins, or receptors following activation of hematopoietic cells. As described in the Tu *et al.* publication (*Blood*, 2001, 99(7):2028-2038, previously submitted), of which Dr. Kerr is a co-author, and pages 3-4 of the aforementioned manuscript, formation of these complexes enables SHIP to hydrolyze the 5'-phosphate on PIP3, thus preventing membrane recruitment and activation of pleckstrin homology (PH) domain containing kinases that serve as effectors of PI3K signaling. SIP-110 was also shown to have enzymatic activity by Jefferson *et al.* (*J. Biol. Chem.*, 1997, 272(9):5983-5988, submitted herewith). This activity permits SHIP and SIP-110 to limit the survival, activation, differentiation, and/or proliferation of

hematopoietic cells. Dr. Kerr's laboratory and others have found that SHIP deficiency results in expansion of the HSC compartment *in vivo*. Furthermore, the results described in the manuscript of Exhibit B show that SHIP-deficiency in the HSC of mice enhances HSC proliferation and survival. The published version of the manuscript (Desponts *et al.*, *Blood*, 2006, 107(11):4338-4345) is submitted herewith.

The data in the Tu *et al.* (2001) publication and pages 22-26 of the patent application make clear that the s-SHIP isoform is expressed from an internal site within the SHIP gene in embryonic stem cells (ESC) and HSC, but not in mature hematopoietic cells. The s-SHIP isoform lacks the SH2 domain found in the SHIP isoform whose expression is restricted to the hematopoietic system. Consistent with this structural difference, s-SHIP does not associate *in vivo* with the Shc adapter protein. However, like SHIP, s-SHIP does associate with the major adapter protein Grb2 and gp130 subunit of the LIF and c-kit receptors, but does not require tyrosine phosphorylation to do so (page 29, lines 7-15 of the patent application).

Kavanaugh *et al.* (*Curr. Biol.*, 1996, 6(4):438-445, submitted herewith) cloned and characterized the human SHIP isoform, SIP-110, that lacks an SH2 domain (GenBank accession number U50040). Kavanaugh *et al.* (1996) and Jefferson *et al.* (1997) also confirmed that SIP-110 binds with Grb2 and hydrolyzes PIP3, likely preventing PIP3 accumulation to significant levels (page 443, first column of the Kavanaugh *et al.* publication). As summarized by Dr. Kerr,

> Thus, like SHIP, SIP-110 opposes PI3K and, thus, PI3K-effector pathways, which control cell proliferation and/or survival. My laboratory's analysis of the structure and the genomic location of the first exon of SIP-110, which is provided in Figure 7 and paragraph [0070] at pages 33-34 of the patent application, confirmed that SIP-110 is the human homologue of s-SHIP. In view of the characterizing data obtained for SIP-110 and s-SHIP in the patent application and the scientific literature at the time the patent application was filed, it is reasonable to extrapolate between these two homologues. Furthermore, based on the data available for SIP-110 and s-SHIP in the patent application and the scientific literature at the time the patent application was filed, it is reasonable to conclude that s-SHIP/SIP-110 are utilized by ESC and HSC to oppose PI3K, thereby regulating cell proliferation and/or survival. Paragraph 4, page 3, of the Declaration (emphasis added).

As described in the aforementioned manuscript, Dr. Kerr's laboratory has demonstrated that $SHIP^{-/-}$ mice generated using the Cre-lox method have an expanded HSC compartment. The Cre-lox

method involves introducing loxP target sequences into the gene to be deleted, and engineering expression of the Cre recombinase enzyme under the control of a tissue-specific promoter. Thus, the enzyme is expressed only in the desired tissue, and it deletes the gene of interest via the loxP target sites. As indicated at page 16 of the manuscript, $SHIP^{-/-}$ mice were generated by deletion of the promoter and first exon of SHIP.

In contrast, RNAi affects the abundance of RNA and in turn, the abundance of protein. The applicants gratefully acknowledge the Examiner's indication at page 7 of the Office Action that the steps needed to make and use shRNA were known in the art at the time the subject application was filed. As explained at page 4, paragraph 6 of Dr. Kerr's Declaration, "RNAi can be used to create 'hypomorphs' (having reduced or weakened gene function) as well as 'complete silencing' (emphasis added). Potency and specificity of gene silencing are the major advantages of the RNAi methodology over other nucleic acid-based gene targeting approaches. This is evidenced by the following scientific reports and review papers published before and after the applications filing date, which are submitted herewith: Diallo *et al.*, "Long endogenous dsRNAs can induce complete gene silencing in mammalian cells and primary cultures", *Oligonucleotides*, 2003, 13(5):381-392; Scherr *et al.*, "Inhibition of GM-CSF receptor function by stable RNA interference in a NOD/SCID mouse hematopoietic stem cell transplantation model", *Oligonucleotides*, 2003, 13(5):353-363; Bot *et al.*, "Lentiviral shRNA silencing of murine bone marrow cell CCR2 leads to persistent knockdown of CCR2 function *in vivo*", *Blood*, 2005, 106(4):1147-1153; Soutschek *et al.*, "Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs", *Nature*, 432:173-178; McCaffrey and Kay, "RNA interference gets infectious", *Gene Therapy*, 10:1205; Kim, "RNA interference in functional genomics and medicine", *J. Korean Med. Sci.*, June 2003, 18:309-318; Sandy *et al.*, "Mammalian RNAi: a practical Guide", *BioTechniques*, 2005, 39:215-224; Lenz, "The RNA interference revolution", *Brazilian Journal of Medical and Biological Research*, 205, 38:1749-1757; and Zhou *et al.*, "RNAi technology and its use in studying the function of nuclear receptors and coregulators", *Nuclear Receptor Signaling Atlas*, September 2003, 1:e008.

Given the state of the art as demonstrated by the scientific publications submitted herewith and previously, and the information provided in the patent application and the experimental results

obtained therewith, one of ordinary skill in the art can target and reduce expression of s-SHIP/SIP-110 without resort to undue experimentation. Furthermore, as stated by Dr. Kerr,

> ... based on the experimental results in Figure 8 of the patent application, and the state of the art of RNAi technology at the time the patent application was filed, one of ordinary skill in the art would reasonably expect that sufficient s-SHIP/SIP-110 knockdown could be achieved to induce proliferation, growth, and/or survival of ESC and HSC. Paragraph 7, page 5, of the Kerr Declaration (emphasis added).

As the Examiner has acknowledged, consideration is to be given to post-filing date evidence (*e.g.*, Declarations and Exhibits) offered by the applicants to show that the claimed invention works provided that the evidence is consonant with the teachings of the specification as filed. In making this determination, the Examiner is to compare the materials and methods used in the experiments of the Declaration and Exhibits with those taught in the application to make sure that they are commensurate in scope. This means that the Examiner is to confirm that the experiments used the guidance in the specification as filed and what was well known to one of skill in the art (MPEP §2164.05). Thus, the requirement of consonance between the submitted evidence and the teachings of the specification is not evaluated in a vacuum. Rather, the determination is to be made from the standpoint of those of ordinary skill in the art, and the knowledge possessed by them (those skilled in the pertinent arts of stem cell biology, cell signaling, and RNAi, for example). A disclosure in an application, to be complete, must contain such description and details as to enable any person skilled in the art or science to which the invention pertains to make and use the invention as of its filing date. *In re Glass,* 492 F.2d 1228, 181 USPQ 31 (CCPA 1974). As iterated in MPEP 608.01(p), the prior art setting may be mentioned in general terms. It is "the essential novelty, the essence of the invention, [that] must be described in such details, including proportions and techniques, where necessary, as to enable those persons skilled in the art to make and utilize the invention."

The enablement requirement of 35 U.S.C. §112, first paragraph, does not require that the applicants reinvent the wheel. There is no need to inform the layman nor disclose what one of ordinary skill in the art already possesses.

> Paragraph 1 permits resort to material outside of the specification in order to satisfy the enablement portion of the statute because it makes no sense to encumber the specification of a patent with all the knowledge of the past concerning how to make

> and use the claimed invention. One skilled in the art knows how to make and use a bolt, a wheel, a gear, a transistor, or a known chemical starting material. The specification would be of enormous and unnecessary length if one had to literally reinvent and describe the wheel. *Amtel Corporation v. Information Storage Devices, Inc.*, 198 F.3d 1374; 53 USPQ2d 1225 (Fed. Cir. 1999).

At page 5, lines 12-15, the Office Action makes reference to claims 41 and 48, which recite "inducing the stem cells to differentiate." Page 6 of the Office Action indicates that Shah *et al.* (1996), White *et al.* (2001), Watt *et al.* (2000), Lee *et al.* (2000), Lumelsky *et al.* (2001), and Appendix D of Stem Cells: Scientific Progress and Future Directions do not indicate a role of s-SHIP or SIP-110 in differentiation/proliferation in the stem cells described in those references. By this Amendment, the applicants have cancelled claims 41 and 48, rendering this aspect of the rejection moot. The applicants' remarks above and the Declaration under 37 C.F.R. §1.132 submitted herewith address the role of s-SHIP and SIP-110 in the proliferation, growth, and/or survival of ESC and HSC.

When taken with the other experimental evidence provided in the subject application and submitted with the applicants' previous responses, it is clear that: (1) one of ordinary skill in the art would expect that s-SHIP/SIP-110 deficiency would increase proliferation of mouse/human HSC and ESC; and (2) sufficient s-SHIP or SIP-110 deficiency can be induced in HSC and ESC using shRNA to achieve the expanded phenotype, without resort to undue experimentation.

The applicants respectfully submit that the subject specification contains sufficient information to enable one of ordinary skill in the pertinent art to make and use the claimed invention without undue experimentation. Accordingly, the applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

In view of the foregoing remarks and amendments to the claims, the applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

The applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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Attachments: Request for Continued Examination
Declaration under 37 C.F.R. §1.132 by Dr. Kerr
Jefferson *et al.*, *J. Biol. Chem.*, 1997, 272(9):5983-5988
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